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Regulation of microRNAs

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PREFACE

When in 2001 the first draft versions of the human genome revealed that there were no more than 25,000 human genes, much soul searching resulted. How could a complicated human being develop and function with a gene set not much bigger than that of a worm, *Caenorhabditis elegans*?

Although the meaning of 'gene counts' is debatable when a single gene can give rise to a multitude of different gene products (and when in fact much of the 'inter-genic' genome appears to be transcribed), the apparent conundrum highlighted the importance of gene regulation in making complex organisms. It thus appears particularly appropriate that it was also in 2001 that microRNAs (miRNAs) were finding their way into the limelight. These regulatory RNAs, named for their small size of some 22 nucleotides, had been discovered in 1993 in *C. elegans*, but were initially considered a worm oddity and largely ignored. It was only when small RNA cloning efforts started to reveal hundreds of different miRNAs in a typical animal or plant genome that they were widely noticed. Today, it appears that hardly any cellular or developmental pathway has escaped the control that miRNAs exert by silencing target mRNAs through an antisense mechanism. Accordingly, miRNAs dysregulation contributes to numerous diseases, most notably diverse cancers.

Given this pervasiveness and importance of miRNA-mediated gene regulation, it should come as little surprise that miRNAs themselves are also highly regulated. However, the recent explosion of knowledge on this topic has been remarkable, providing a primary motivation for publication of this book. As miRNAs are transcribed by RNA polymerase II, the enzyme that also generates mRNAs, it was perhaps not unexpected that miRNA transcription would be subject to regulation, and we have willfully omitted this aspect from this monograph. However, what has been unexpected is the extent of post-transcriptional regulation of miRNAs that is illustrated in this book.

In the first chapter, René Ketting provides the background against which all of the regulatory processes occur by revealing the complex biogenesis and function of miRNAs and the related siRNAs. Akiko Hata and Brandi Davis then describe how SMAD proteins, generally known for their function in controlling transcription, reveal another side in regulating the processing of certain primary miRNA (pri-miRNA) transcripts by the RNase Drosha. Drosha-mediated processing of pri-miRNAs into the short precursor

miRNAs (pre-miRNAs) is also modulated by the RNA-binding proteins hnRNP A1, as discussed by Javier Caceres and colleagues and KSRP (Michele Trabucchi et al). Whereas SMADs, hnRNP A1 and KSRP promote processing of specific pri-miRNAs, estrogen receptor alpha represses this biogenesis step as related by Shigeaki Kato and colleagues. Robinson Triboulet and Richard Gregory further reveal that the pri-miRNA processing complex undergoes autoregulation.

KSRP not only promotes processing of pri-miRNAs, but also the subsequent cleavage of pre-miRNAs by the RNase Dicer. Conversely, Lin28 was found to repress pri-miRNA as well pre-miRNA processing as discussed by Nicolas Lehrbach and Eric Miska. This inhibition involves 3' end uridylation of the pre-miRNA. Another RNA modification that occurs on miRNAs is adenosine-to-inosine editing, and Mary O'Connell and colleagues critically evaluate its incidence and how editing affects processing and functionality of miRNAs.

Gregory Wulczyn and colleagues discuss the Trim-NHL protein family whose members utilize diverse mechanisms to regulate miRNA levels and activity both positively and negatively. Nicole Meisner and Witold Filipowicz review HuR, an RNA-binding protein that regulates mRNAs through a number of mechanisms, including at least one instance in which HuR reverses miRNA-mediated mRNA silencing.

Finally, although mature miRNAs have long been viewed as highly stable molecules, miRNA degradation pathways have now been identified in plants and algae, as revealed by Heriberto Cerutti and Fadia Ibrahim, and in animals, as discussed by us.

Even if this monograph cannot strive to be comprehensive in a field developing at such an amazing pace, I hope that the examples provided here will serve to illustrate the diversity of mechanisms regulating miRNAs, as well as highlight some unifying themes, particularly among the mechanisms regulating miRNA biogenesis. Undoubtedly, many more examples of regulation of miRNAs remain to be discovered and mechanistic details on known pathways to be revealed, promising an exciting future to this field of research.

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ABOUT THE EDITOR...

HELGE GROßHANS, PhD is a research group leader at the Friedrich Miescher Institute for Biomedical Research (FMI), which is part of the Novartis Research Foundation in Basel, Switzerland. His main research interests are in the developmental function, regulation, and mechanism of action of microRNAs. He received his PhD from the University of Heidelberg, Germany and did his postdoctoral training at Yale University, USA. He is the winner of a 2009 ERC Award.

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CONTENTS

CHAPTER 1

microRNA BIOGENESIS AND FUNCTION
An Overview

René F. Ketting*

Abstract: During the last decade of the 20th century a totally novel way of gene regulation was revealed. Findings that at first glance appeared freak features of plants or *C. elegans* turned out to be mechanistically related and deeply conserved throughout evolution. This important insight was primed by the landmark discovery of RNA interference, or RNAi, in 1998. This work started an entire novel field of research, now usually referred to as RNA silencing. The common denominator of the phenomena grouped in this field are small RNA molecules, often derived from double stranded RNA precursors, that in association with proteins of the so-called Argonaute family, are capable of directing a variety of effector complexes to cognate RNA and/or DNA molecules. One of these processes is now widely known as microRNA-mediated gene silencing and I will provide a partially historical framework of the many steps that have led to our current understanding of microRNA biogenesis and function. This chapter is meant to provide a general overview of the various processes involved. For a comprehensive description of current models, I refer interested readers to the reviews and primary literature references provided in this chapter and to the further contents of this book.

INTRODUCTION: PTGS IN PLANTS AND SMALL RNAs

In the early 90s a number of papers were published that revealed an activity in Tobacco and Petunia plants that was triggered by repetitive transgenic DNA and that resulted in the silencing of that DNA and any other DNA bearing significant homology to the trigger (cosuppression).[1,2] At least part of these phenomena acted downstream of transcription, through destabilization of mRNA and hence was named "Post-Transcriptional Gene Silencing" (PTGS). The molecular trigger of this phenomenon was not clear, although

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it was speculated that "aberrant" RNA or double stranded RNA (dsRNA) were good candidates for priming PTGS. Although aberrant RNAs still play an important role in many models on RNA-mediated silencing events in plants, for example as templates on which dsRNA is synthesized, we now know that dsRNA is indeed in most cases the primary trigger. Furthermore, in 1999 a landmark paper from David Baulcombe and colleagues identified small RNA molecules as potential "specificity determinants" in PTGS.[3] This hypothesis has turned out to be absolutely correct and the identification of this type of small RNA species helped to lay the basis for an outburst of research activity on RNA-based silencing processes in the years that followed.

RNAi

In 1998, double-stranded RNA (dsRNA) was first described as a very potent and specific agent for gene silencing in *C. elegans*. The term RNA interference was coined to refer to the described silencing effects, a term that is now usually abbreviated to RNAi.[4] This ground-breaking work, published by Craig Mello, Andrew Fire and their colleagues, was awarded the Nobel Prize in 2006. Mello and Fire noted that RNAi targets exonic regions in RNA and leads to decreased RNA levels, consistent with a model in which RNAi leads to sequence specific mRNA destabilization, as had been found for cosuppression in plants. It was also noted that dsRNA could very well be a trigger in plant cosuppression, since inverted repeat sequences had been described as very potent triggers of PTGS. Soon after this paper, RNAi-like processes were identified in numerous other systems.[5] Biochemical experiments in *Drosophila* started to reveal a mechanistic framework of RNAi,[6,7] while genetics in *C. elegans* was revealing endogenous functions for RNAi and genes required for it.[8,9] It appeared that RNAi could mechanistically be roughly divided into two steps: an initiation step and an effector step (Fig. 1).[10] In the initiation step small RNAs are generated from the dsRNA trigger; in the effector step these small RNAs guide an Argonaute protein-containing complex named RNA-Induced Silencing Complex (RISC) to cognate mRNAs. The realization that small RNA molecules (then named siRNAs, for short interfering RNAs), like those described by Baulcombe in Tobacco plants undergoing PTGS, rather than long dsRNA molecules provided the sequence specificity of the whole process,[7,11] enabled efficient RNAi also in mammalian cells.[12] This provided a highly efficient way to perform reverse genetic experiments in cell culture systems, a finding that has revolutionized research on mammalian cells.

DICER

Dicer, the enzyme that generates siRNAs from dsRNA, was identified in 2001.[13] This enzyme contains two RNase III active sites, a so-called PAZ domain (named after three proteins in which this domain was first recognized: Piwi, Argonaute and Zwille), a helicase domain and a dsRNA-binding domain. It binds to the ends of dsRNA substrates and introduces a staggered double-stranded break further along the dsRNA.[14] The catalytic activity is very characteristic and always leaves a 3′-hydroxyl group, a 5′-phosphate group and a two base overhang at the 3′ end. The length of the small RNA generated can vary, but usually is between 20 and 25 bases. Within one organism, different Dicer

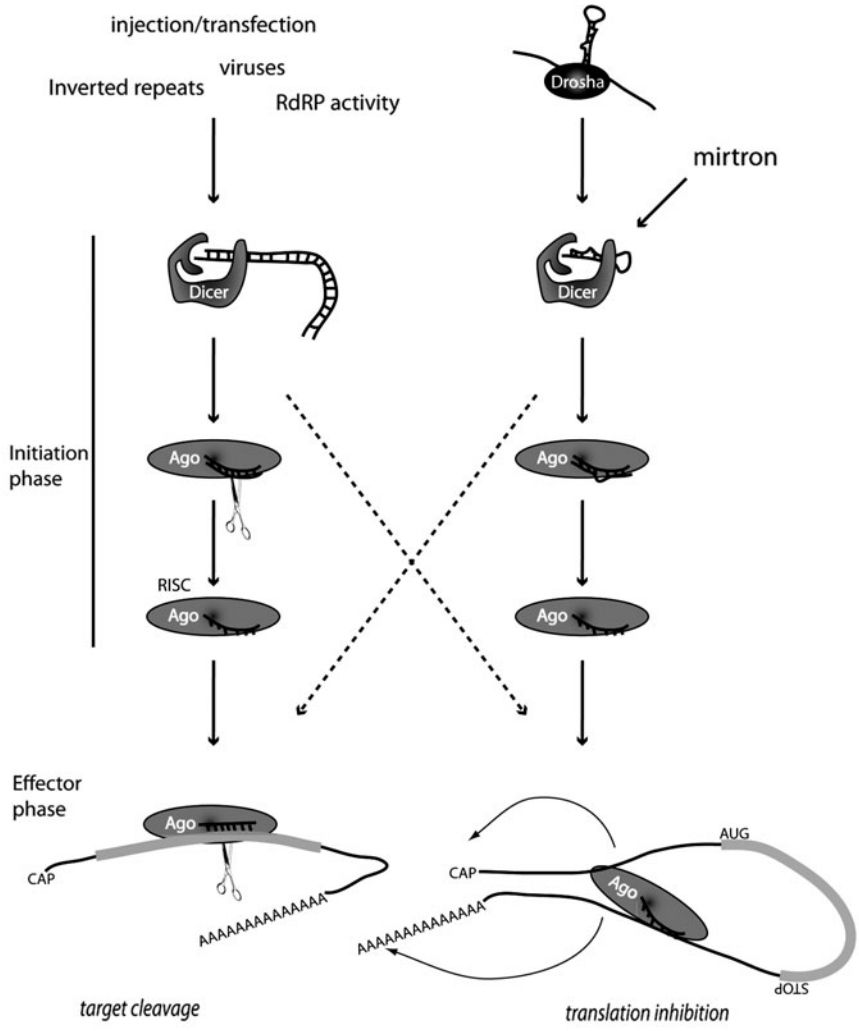

Figure 1. Schematic comparison between RNAi and miRNA mechanisms. For a more detailed scheme of miRNA action see Figure 2. "RdRP activity" refers to RNA-dependent RNA polymerase activity that in plants and yeast can turn ssRNA into dsRNA that is subsequently cleaved by Dicer. Likely, this is a major source for the dsRNA trigger in PTGS. The scissors indicate passenger strand and target cleavage. The dashed lines crossing from RNAi to miRNA and vice versa indicate that the separation between these pathways is not absolute: side effects from siRNAs in RNAi experiments can be triggered through miRNA like activities and miRNAs are capable of inducing target cleavage if presented with a properly matching target RNA. The type of silencing induced is also strongly dependent on the sub-type of Argonaute protein involved.

genes can be present, each encoding a protein generating rather specific subsets of small RNA products.[15] Mammals, however, only have one Dicer gene.